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Growth Control and Differentiation in Mammary Epithelial Cells

by Flavia Borellini* and Takami Oka*

Growth and differentiation of the mammary gland are controlled by various hormones and other environmental factors. The role of hormones and growth factors in mammary development is discussed with regard to animal species, physiological stages, and the various experimental systems *in vitro*. In the female embryo, mammary morphogenesis is induced by the mesenchyme and is hormone independent, whereas androgens cause the partial necrosis of mammary epithelium in the male. Ductal growth during adolescence requires estrogen and prolactin or growth hormone. During pregnancy, progesterone participates in the development of the lobuloaveolar structure of the gland. After parturition, changes in the hormonal environment lead to production and secretion of milk. Proliferation and differentiation of mammary epithelium can be induced in culture systems. Insulin and epidermal growth factor (EGF) stimulate mammary cell proliferation *in vitro*. EGF is required for the optimal growth of the mammary gland during pregnancy. EGF also appears to play an important role in mammary tumorigenesis in certain mouse strains. Production of milk proteins can be induced *in vitro* by the synergistic interactions of prolactin, insulin, and glucocorticoids and is inhibited by EGF and progesterone. Complete or partial sequencing of several milk protein genes and comparative analysis have led to identification of a sequence of high homology and conservation in the 5′ flanking region that is likely to be involved in the regulation of milk protein gene expression.

Introduction

In recent years, biomedical studies have focused on understanding the mechanisms regulating cell growth and differentiation. For this purpose, the mammary gland serves as a very suitable experimental system because of its characteristic pattern of morphological and functional development.

Most tissues and organs undergo massive growth in the early stages of development, i.e., during embryogenesis and in the early period of postnatal life. By contrast, mammary tissue expresses its maximum growth potential after the animal has reached maturity, that is, following the onset of pregnancy and during lactation. Moreover, this growth potential is maintained throughout the reproductive life of the animal, and, to some extent, even thereafter. The cycle of proliferation-differentiation-regression is repeated at each gestation, and can be reproduced in culture systems *in vitro*. The characteristic physiological pattern of mammary gland development is regulated by several factors; endocrine control is the most obvious and perhaps the best understood, but several studies have provided evidence that paracrine, autocrine, and intracellular factors, as well as the extracellular matrix, can also affect the growth and differentiation processes.

The availability of molecular biological techniques has given investigators a new possible approach to the understanding of the complex mechanisms of regulation of mammary gland function. Studies on the structure and expression of milk protein genes have provided valuable information to the overall picture. A deeper understanding of how growth and differentiation of the mammary tissue are regulated could complement the knowledge of developmental processes and also provide invaluable information for clinical treatment and prevention of mammary cancer.

Several excellent reviews (*1-7*) giving an extensive overview of the growth and development of the mammary gland have been published. In this review, we have focused on the recent progress made in this field, with special attention to the interactions between the different regulatory factors and their mechanisms of action.

*Laboratory of Molecular and Cellular Biology, National Institute of Diabetes, Digestive and Kidney Diseases, National Institutes of Health, Bethesda, MD 20892.

Address reprints to Takami Oka, Laboratory of Molecular and Cellular Biology, National Institute of Diabetes, Digestive and Kidney Diseases, National Institutes of Health, Bethesda, MD 20892.

Outline of Mammary Gland Development

The mammary tissue from different species has a very similar structural organization, although hormonal re-

quirements for the growth and expression of the specific function of the gland vary among various strains or species. The following is a brief account of developmental changes of the mammary gland.

Embryogenesis

The embryonal development of mammary tissue appears to be comparable in all species (*8,9*). In the mouse, the development in the male and in the female follows the same pattern up to about the day 13 of embryonal life; at day 11, an area of raised ectoderm is formed on both sides of the trunk, and the neighbouring ectodermal cells begin to congregate around this area to form the mammary band (Fig. 1A). The migration of the epidermal cells to preferential centers of congregation gives rise to the individual buds (*8,9*) (Fig. 1B).

The critical difference between male and female embryos in the development of the mammary tissue occurs between days 13 and 15. In the female, at day 15 of embryonic life, the mammary bud cells undergo a phase of rapid proliferation, giving rise to the mammary cord, a band of epithelial cells elongated into the mesenchyme but still connected to the epidermis by a collar of ectodermal cells (Fig. 1C). At the end of gestation, the mammary epithelium in the female embryo consists of a cord of cells, embedded in the mesenchyme and partially canalized. The cord opens at the apex, where the future nipple will be formed, and begins to show some branching at the distal end.

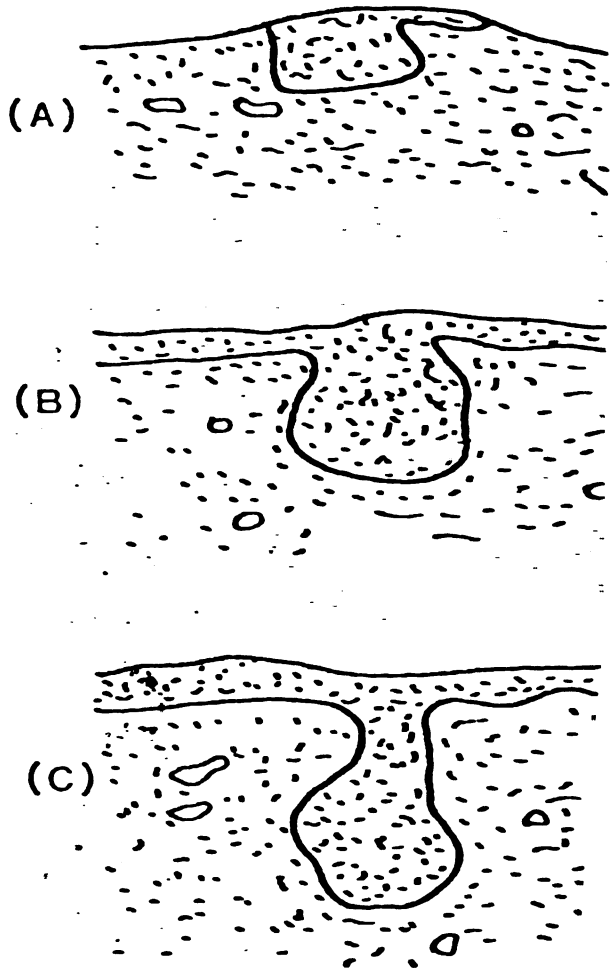

FIGURE 1. Formation of the mammary cord in the mouse embryo between days 11 and 15 of gestation. (*A*) Mammary band, day 11; (*B*) mammary bud, day 12-13; (*C*) mammary cord, day 15.

In the male embryo, a series of events takes place between days 13 and 15 as a result of the androgen production by the fetal testes (*10*). The mesenchyme condenses all around the mammary bud, which becomes isolated into the subepidermal tissue. The bud eventually becomes a rudimentary epithelial cord, but no further development occurs. In the mouse, the mesenchymal condensation leads eventually to the partial or total destruction of the mammary bud; in rat and rabbit embryos the necrosis of the mammary rudiment does not occur.

From Birth to Maturity

The prepubertal development of the mammary gland occurs in two distinct phases. Up to day 22 to 23 of life, the development of the gland is mainly due to an increase in connective tissue and deposition of fat. The increase in size parallels the increase in body weight, and is therefore referred to as isometric growth; it does not seem to depend on hormones. During the 2 weeks preceding puberty, the mammary gland grows at a rate about three times greater than that of the body weight. This phase is referred to as allometric growth, and can be prevented by ovariectomy (*2*).

Sexual maturation in the mouse occurs during week 4 to 6 of postnatal life. During this phase the mammary epithelium in the female grows extensively and extends throughout the fat pad. Very few alveoli are formed at this time; the growth pattern is three-dimensional and can be simulated in cultures of mammary epithelium into a collagen matrix (*11*). Elongation of the ducts occurs as a result of rapid growth in end buds, which, by turning and branching, give rise to the characteristic tree-shaped pattern of the mammary ductal system. The end bud tip is covered with a monolayer of epithelium, the cap cells, which are characterized by a relative lack of specialized features. These cells have been defined as a stem cell population; as the duct elongates, the cap cells gradually differentiate to provide new myoepithelial cells for ductal morphogenesis and elongation (*12*). In human breast tissue, myoepithelial cells also originate from a precursor population within the epithelium after a number of cell divisions (*13*). During the different stages of mouse mammary gland development, myoepithelial cells undergo several changes in size and shape (*14*). The stroma surrounding the elongating end bud shows a significant increase in DNA synthesis (*15*).

As the mouse reaches sexual maturity at 6 to 8 weeks of age, the development of the ductal structure stops, in spite of the presence of the major hormonal stimuli (i.e., estrogen produced by the ovaries), for ductal growth. Some residual mitotic activity is observed at the end buds

in the mature mouse gland during estrus (*16*). At this stage, the mammary gland shows ductal ramifications throughout the mammary fat pad; however, a clear zone of fat pad remains between the ducts. This interductal space will eventually be occupied by lobules during pregnancy.

Pregnancy

Following the onset of pregnancy, cell replication resumes. During the first period, ductal growth occurs extensively, and the ducts undergo further branching. The interstitial adipose tissue disappears progressively, and proliferating epithelial cells fill the interductal spaces. During the middle and late period of pregnancy, epithelial cells continue to multiply to form the lobuloalveolar structure. As pregnancy approaches term, the alveoli start to show secretory activity, and the epithelial cells become distended, with a granular cytoplasm (*17,18*) (Fig. 2). Increased vascularization of the gland and loss of fat in fat pad cells seem to be induced by the proliferating epithelial cells. In rat mammary gland, the proliferative activity is also higher during estrus and pregnancy than during nonestrus and lactation (*19*).

Lactation

Functional differentiation of mammary epithelial cells culminates in lactogenesis. Lactating tissue shows the typical features of a secretory epithelium. Cells are highly polarized, both functionally and morphologically, are connected by tight junctions, and the surfaces that face the cavity of the alveoli are covered with microvilli. A large part of the cytoplasm is occupied by rough endoplasmic reticulum; the well-developed Golgi apparatus is located in the apical region. Small droplets of fat are present all over the cytoplasm (Fig. 2). The epithelium and the surrounding myoepithelial cells are enclosed by the basal lamina, which provides a boundary between the epithelium and the stromal tissue.

In the mouse, milk yield is maximum at day 15 of lactation. After day 15 a sharp drop occurs, followed by a decrease in the synthetic activity of the gland and in cell population. When lactation is extended by substituting younger pups to the original litter at day 15, cell number and differentiation are maintained even though at a lower degree (*20*).

Involution

Natural weaning is usually a gradual process, and the progressive decrease in the suckling stimulation results in involution of the gland. The major changes of involution take place in the epithelial cells, which begin to show large vacuoles containing casein micelles and large fat droplets. As weaning progresses, more vacuoles appear, enclosing a variety of subcellular organelles such as mitochondria and fragments of endoplasmic reticulum. Hydrolytic enzymes are released by the fusion of lysosomes with the vacuoles. This autophagic process leads to cell depletion and lysis. The cell residues are ingested by macrophages present in a quite large number in the tissue at this stage. However, not all of secretory epithelial cells are destroyed; some of them undergo a partial autophagocytosis but survive. The myoepithelial cells surrounding the alveoli are usually preserved, although their shape changes from starlike to a more compact form.

Determinants of Mammary Gland Development *In Vivo*

The regulation of mammary gland morphogenesis *in vivo* is a very complex phenomenon that involves both hormonal stimuli and environmental factors. To simplify the exposition, we have grouped under the section "Hormonal Determinants" all the factors acting in an endocrine, autocrine, or paracrine fashion through a specific receptor. Several factors of mesenchymal origin seem to affect epithelial growth in a paracrine fashion. We will limit our discussion of this subject to generic information; for an extensive treatment, we refer the reader to the specific papers in this journal.

Hormonal Determinants

In the female, mammary embryogenesis is induced by the underlying mesenchyme and is hormone independent. Destruction of the ovaries with X-ray radiation in the 13-day embryo does not affect mammary development (*21*). In the male, the mesenchyme-directed development is interrupted at day 13, when mammary rudiments acquire sensitivity to androgens; after day 15 they lose hormone responsiveness. Destruction of the fetal testes with X-rays on day 13 leads to mammary development similar to that in the female (*21*).

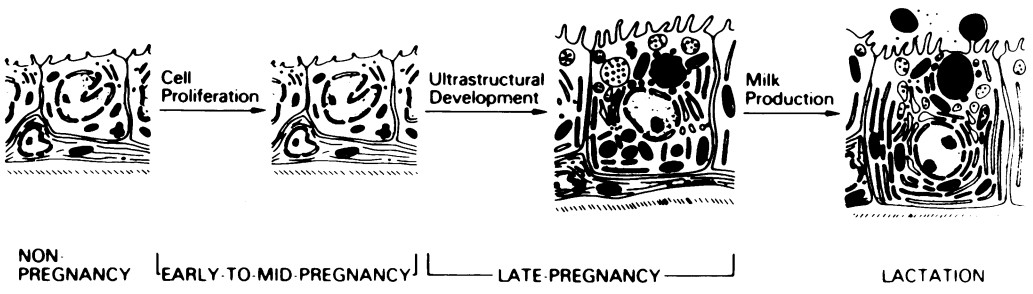

FIGURE 2. Development of the mammary epithelial cell during pregnancy and lactation.

Cell replication during adolescence is hormone dependent (*22,23*). Ductal growth requires estrogen and either growth hormone or prolactin; however, neither type of hormone is effective alone. Extensive growth can occur in the absence of glucocorticoid, but maximal growth requires the steroid. Ductal growth appears to be independent of progesterone, thyroid hormones, and the thymus (*7,24*). Insulin is not involved in ductal growth *in vivo*, as the cells in virgin mice are not responsive to the hormone (*25*). In some species, as in the rat, the requirements for ductal growth vary among the different strains (*2,26,27*).

17-β-Estradiol is an absolute requirement for ductal growth, and when administered to a mouse it can enhance DNA synthesis in the mammary epithelium. It has been reported that the effect of estrogen on DNA synthesis begins approximately 12 hr after treatment (*28*), and maximal stimulation is reached at 48 hr. Autoradiographic studies have shown that the increase in thymidine incorporation is mostly restricted to the adipose and connective tissues during the first 24 hr; at 48 and 72 hr the epithelium undergoes extensive replication. Thus estrogen exerts its effect initially on stromal or fat pad cells (*28*). It would be interesting to study the relationship of this initial response to the subsequent proliferative response of the epithelium.

There seems to be a synergism between estradiol and cAMP in stimulating the growth of mammary epithelium (*29*); however, it is not clear whether the presence of ovarian hormones is necessary for cAMP to affect growth and morphogenesis of the gland because of the conflicting reports (*30,31*).

When the animal reaches sexual maturity, the mammary tissue enters a phase of dormancy during which only a modest replicative activity is observed at the end buds during estrus (*16*). However, estriol and progesterone administered daily to mature mice stimulate DNA synthesis and mitosis in resting mammary epithelium (*32*).

The maintenance of duct cells has different hormonal requirements from ductal growth; apparently pituitary hormones are not necessary when either adrenal or ovarian activity is present (*7*).

Lobuloalveolar growth, which occurs mainly during pregnancy, requires estrogen, either growth hormone or prolactin, and progesterone (*1,33,34*). Adrenal steroids and thyroxine seem to enhance the formation of lobules (*7,35*). Even though mouse mammary adipose and connective tissues have high affinity binding sites for estrogens and progesterone, the largest amount of progesterone receptors is present in the epithelial component (*36*). Moreover, regulation of progesterone receptor concentration by estrogen and its changes during the different developmental phases appears to be restricted to the mammary epithelium (*36*). Ovarian steroids are also required for maintenance of alveoli.

Onset of lactation coincides temporally with a decrease in progesterone level and elevation of prolactin and glucocorticoids. Whether the trigger for the onset of milk production is due to the decrease in circulating progesterone or to the increase in a positive stimulus (prolactin and glucocorticoid) is still an open question. However, lactating mammary tissue has no detectable progesterone receptors (*37,38*), and, in fact, progesterone is unable to inhibit established lactation. On the other hand, administration of estrogen and progesterone to a lactating rat stimulates the rate of mammary cell replication that is similar to that observed during pregnancy (*19*).

Several lines of evidence support a role of epidermal growth factor (EGF) in mammary epithelial proliferation during pregnancy. The level of EGF receptors varies during the physiological stages of the gland: it is relatively low in the virgin and lactating glands, but increases during pregnancy and reaches a peak on day 10 of gestation (*39*).

The levels of EGF in the submandibular gland and plasma increase substantially during pregnancy (*40*). As sialoadenectomy results in marked decrease in the plasma EGF, it appears that the submandibular gland serves as a major source of circulating EGF. Earlier studies have indicated that the amount of EGF in the submandibular gland is increased by androgens (*41*) and progestins (*42*). As the level of these steroid hormones rises during pregnancy (*43*), it is possible that the increase in submandibular EGF is elicited by the action of androgens and/or progestin. In addition, as will be described later, physiological concentrations of EGF stimulate proliferation of mammary epithelial cells in culture.

Further evidence for the involvement of EGF in mammary gland development during pregnancy is provided by the work of Okamoto and Oka (*44*); the mammary glands of lactating mice that have been sialoadenectomized pregestationally are smaller and produce a smaller amount of milk compared to that of sham-operated controls. As a consequence, sialoadenectomized mothers cannot properly support all the litter, and thus a substantial number of pups die within the first week after birth. EGF-replacement therapy given to the sialoadenectomized mice during pregnancy restores the growth of the gland, milk production, and pup survival rate (*44,45*).

Thyroid hormones seem to have a role in the maintenance of alveoli and ductal branching in the involuting gland; the mammary gland of C3H mice treated with 2-thiouracil to repress thyroid function during involution shows less ductal branching and no alveoli (*35*).

Reinitiation of growth in senescent mammary epithelium has been obtained by application of implants that release cholera toxin (a cAMP-active agent) in the gland. This implies that the growth potential is maintained in the epithelium throughout the life span and that the change in the hormonal milieu is responsible for the alterations in growth patterns during the different physiological stages (*46*).

The Role of Stromal Components

Two different kinds of mesenchymal cells are involved in the morphogenesis of mammary gland epithelium of the mouse: fat pad precursor cells and fibroblasts. The specific interaction between fat pad precursor cells and epithelial cells seems to be important for determining the

shape of the ductal branching structure in the female embryo.

The mesenchyme also plays a key role in the interruption of development that occurs in the male embryo: it has been identified as the specific target tissue for testosterone action, although its response to the hormone requires the presence of the mammary epithelium (*47*). More recent studies suggested that the mammary epithelium could induce the formation of androgen receptors in the surrounding mesenchyme, thus controlling the development of androgen responsiveness in the tissue (*48,49*).

During the massive development that occurs at sexual maturation, epithelial-adipocyte interactions appear to be required for the formation of end buds and the subsequent morphogenesis of fully structured mammary ducts. White fat tissue from a different origin other than mammary fat can support ductal growth (*50,51*).

For the epithelium to become responsive to lactogenic hormones, certain environment-associated events must occur. In co-culture systems of mouse mammary epithelium and adipocytes or other fibroblasts, only 3T3L1 and 3T3C2 cells could support casein synthesis in the presence of the lactogenic stimuli, insulin-cortisol-prolactin (*52*). There was evidence of specificity of the epithelium-stroma interaction during this stage because Swiss 3T3 cells, newborn foreskin fibroblasts, substrate-attached materials, and tissue culture plastic did not support casein synthesis (*52*). These results indicate that the acquisition of hormone sensitivity and hormone-dependent differentiation of mammary epithelium are influenced by the stromal milieu. In cultured mouse mammary explants, stroma cells appear to support the epithelial differentiation partly by providing type I collagen, an essential component of extracellular matrix (*53*).

Biochemical Markers of Differentiation *In Vitro*

Differentiation of mammary epithelium is usually evaluated by measuring the capacity of the mammary tissue to produce the major milk proteins, such as the casein family and α-lactalbumin.

Before the development of molecular biological techniques, casein production was evaluated by immunoprecipitation with a polyclonal antiserum (*54,55,56*), and α-lactalbumin was measured by enzymatic assays (*57,58*) or immunoprecipitation with the antibody (*59,60*). The more recent approach is focused on studying transcription and stability and accumulation of specific messenger RNAs for milk proteins; cDNAs are available for some of the known caseins, α-lactalbumin, and whey acidic protein (*61–65*).

The α-lactalbumin genes from man and rat have been completely sequenced (*66*); partial sequences are available for several casein genes (*67*). Very recently, Hall and coworkers have compared the promoter regions of two α-lactalbumin and five casein genes and have found a region of high conservation and homology spanning from 140 to 110 in all the sequences examined (*66*). The 5′ flanking region, which includes the consensus sequence, is likely to be a locus for hormonal regulation of milk protein genes expression (*66*). The exact meaning of this conserved sequence will be elucidated by studying the expression of milk protein genes in transfected cell lines and transgenic animals.

Growth and Differentiation *In Vitro*

It has been well established that the growth and differentiation of mammary cells can be induced *in vitro*. The *in vitro* systems have provided invaluable information about the morphogenesis, growth, and differentiation of the mammary gland.

The first model system for the study of mammary growth and differentiation *in vitro* was reported in 1957 by Elias (*68*); this mammary explant culture system has been widely used over the years. A whole mammary gland culture system has been established by Ichinose and Nandi (*69*); more recently, several methods for primary culture of mammary epithelial cells have been developed. Several nonplastic substrates, such as collagen gels (*70–75*), extracellular matrix (*76,77*), and L3TL1 adipocytes (*52,78*) have been used for their capacity to support and promote mammary epithelial growth and differentiation. Cell culture offers the opportunity to distinguish the activity of the different cell populations in the mammary gland; whole gland cultures are particularly suitable in studying the structural changes occurring during the various phases of development.

Most of the studies in culture have been performed using mouse mammary tissue from animals in different physiological stages; data obtained from *in vitro* experiments involving mammary glands from several other species such as rat, rabbit, cow, and goat have revealed some differences in the requirements for morphological and functional development (*7*).

Explant Culture

Explant culture, the first system developed, has provided information about the embryogenesis of the mammary gland (*5–7*). Body wall fragments from the mammary region of mouse embryos at 9 to 10 days of gestation have been shown to develop mammary buds when cultured on a mixture of cock blood plasma and chick embryo extract (*79*). Under similar culture conditions, explants with preformed buds developed branched mammary ducts.

In organ culture of mammary tissue from mouse and rat, insulin extends cell viability of explants in serum-free medium and stimulates cell replication (*25*). Addition of glucocorticoid and insulin can induce the development of rough endoplasmic reticulum (*80-82*). The expression of the differentiated phenotype requires the addition of prolactin (*5,7,80–83*). On the other hand, explants from rabbits can synthesize casein in the presence of prolactin alone (*84*).

Whole Gland Culture

Whole gland cultures of immature mouse mammary gland revealed differential hormonal needs of cell subpopulations for maintenance and development. Primary duct cells can be maintained and proliferate without any added hormone, whereas secondary and tertiary ducts require insulin for maintenance and show an enchanced proliferation in the presence of mineralcorticoid and prolactin. Maintenance of end bud cells requires a complex combination of hormones, and proliferative activity of these cells *in vitro* is modest (*85*).

In mouse whole gland cultures, lobuloalveolar growth can be induced by insulin, prolactin, and glucocorticoid or aldosterone, but the animal has to be previously primed with estrogen and progesterone (*69,86,87*); residual amounts of steroids carried over into the culture could account for the independence of lobuloalveolar formation on the added ovarian hormones *in vitro*. On the other hand, rat mammary gland does not require the priming with ovarian steroids (*88*).

Following hormonally induced lobuloalveolar growth, the whole gland in culture responds to the lactogenic hormones by synthesizing casein. After the first round of development, differentiation, and regression in the whole gland in culture, Tonelli and Sorof were able to induce a second complete cycle by supplementing the serum-free medium with EGF (*89*).

Cell Culture

As discussed earlier, several substrates have been tested for their ability to support cell maintenance and proliferation. One of the problems that investigators have encountered in establishing an efficient cell culture system is reproducing the complex stromal-epithelial interactions that are important for mammary growth *in vivo*. In explants and whole gland cultures these interactions are retained, at least to some extent. Differentiation of mammary epithelial cells in culture requires proper substrate such as collagen and extracellular matrix, as well as lactogenic hormones such as insulin, glucocorticoid, and prolactin (*72–74,76*).

The epithelial cells of the rat embryo mammary bud at day 16 are apparently committed as mammary cells, and they respond to the three hormone combination by synthesizing casein (*90*). However, it is still not known when they first acquire this potential. No data are available about mouse mammary cells in embryo, but explant cultures of immature mouse mammary gland can synthesize caseins (*91*) and accumulate lactose synthetase activity in the presence of appropriate hormonal stimuli (*92*). Rat end bud cells grown on collagen matrix with serum and cholera toxin require insulin, glucocorticoid, estrogen, and prolactin to express functional and ultrastructural differentiation (*93*).

In epithelial cell cultures from mature mouse mammary gland, ultrastructural development and differentiation occur in the same hormonal conditions as in the explant cultures. Growth hormone and placental lactogen can replace prolactin in this system (*74,94*).

Epidermal growth factor and glucocorticoids can affect the growth efficiency of rat mammary epithelial cell cultures on different substrates, apparently modifying the rates of type IV collagen synthesis and degradation (*95*). According to McGrath et al., EGF appears to influence the cell type and the colony shape in serum-free culture of normal rat epithelial cells (*96*).

Maintenance of normal human breast tissue in organ culture requires the presence of insulin, cortisol, and prolactin or serum. Addition of progesterone in combination with insulin, cortisol, and prolactin to a serum-supplemented medium provides a better condition for epithelial growth (*97*). According to Hillman and co-workers, serum-supplemented medium enriched with insulin and cortisol can support explants from normal mammary tissue up to 6 months (*98*).

More recently, a serum-free medium containing insulin, cortisol, EGF, and bovine pituitary extract has been found to be capable of supporting the growth of normal human mammary epithelium up to 10 to 20 passages. Substitution of the bovine pituitary extract with prolactin or prostaglandin E1 allows cell replication for 3 to 4 passages (*99*). Stampfer et al. reported an optimal condition for mammary epithelial cell growth in the presence of insulin, cortisol, EGF, and steroids that allowed the culture to be maintained up to 3 months (*100*).

Hormonal requirement for *in vitro* maintenance varies depending on the substratum. Yang et al. report that epithelial cells from human mammary fibroadenomas require EGF and cortisol when cultured in three dimensions into collagen gel matrix, whereas cortisol alone is sufficient for two-dimensional growth on collagen-coated dishes (*101*). In short-term monolayer culture of normal human mammary epithelium, cortisol and insulin stimulate growth; however, the degree of stimulation depends on the culture substratum. Cells organized into clusters undergo either inhibition of growth or terminal differentiation after a few passages; all isolated cells differentiate. The inhibited cells can resume growth when growth units are disrupted. This situation could resemble the one in mammary buds: The inhibited cells might provide pools for subsequent multiple cycles of differentiation (*102*).

Factors Involved in Regulation of Growth and Differentiation *In Vitro*

A very large amount of experimental data are available today; however, the differences between the experimental systems used and the complexity of the regulatory network operating during mammary gland growth and differentiation makes a clear organization of the subject extremely difficult. In this section we try to summarize the recent data concerning some of the effects of the principal agents known to affect mammary development and function *in vitro*.

Insulin

In serum-free culture, insulin stimulates cell replication

but does not seem to be necessary for ductal or alveolar growth (*103–105*). Addition of an adrenal steroid, usually cortisol, to the culture medium induces the epithelial cells to develop the subcellular organelles, such as the rough endoplasmic reticulum, necessary for the synthesis and secretion of milk components (*80–82*). However, it is only in the presence of prolactin that the tissue achieves complete and functional differentiation (*5,7,80–83*).

Lithium ion (*58,106*), as well as sodium orthovanadate (*107*), can mimic the stimulatory effects of insulin on DNA synthesis in explant culture. On the other hand, EGF (*108*) and serum (*109*) appear to be able to replace insulin in the induction of both DNA synthesis and RER formation.

By measuring parameters like glucose-6-phosphate dehydrogenase activity and DNA synthesis, *in vitro* experiments suggested that mouse mammary epithelial cells *in vivo* undergo alternate phases of insulin resistance and sensitivity (*25*). Cells from immature or mature virgin mice are insulin insensitive but become responsive after 1 day in culture, regardless the presence of exogenous insulin. Mammary epithelial cells of pregnant or lactating mice are insulin sensitive, but loose their sensitivity during postlactational involution (*25*). The modulation of insulin sensitivity in mammary tissue could therefore be a key issue in the regulation of mammary development.

Inagaki and Kohmoto have shown that mouse mammary cells possess insulin receptors with a high (K_d = 1 nM) and a low (K_d = 20 nM) affinity, and the amount of the high affinity receptor is higher during the first half of pregnancy (*110*). Direct evidence supports the hypothesis that the tyrosine kinase activity of the insulin receptor is essential for insulin action (*111*).

In culture with cortisol and prolactin, insulin can induce terminal differentiation in mouse mammary explants by enhancing transcription of casein genes (*112*). Neither serum (*109*) nor EGF (*108*) can substitute for insulin in the synthesis of milk proteins *in vitro*. Lithium also cannot substitute for insulin in the induction of the terminal mammary differentiation, although the sensitivity of the mammary cells to lithium ions is greater in midpregnant than in virgin or lactating animals (*58,106*).

A further proof of the involvement of insulin in terminal differentiation is the fact that tissue from virgin mice can undergo terminal differentiation *in vitro* only after it has acquired sensitivity to insulin (*25,103*).

Cortisol

The concentration of cortisol required for maximal expression of casein and α-lactalbumin is remarkably different. Optimal casein production occurs in the presence of 3 μM cortisol, whereas 30 nM is sufficient for α-lactalbumin. Moreover, cortisol concentrations ranging from 300 nM to 3 μM cause progressive inhibition of α-lactalbumin accumulation (*55,113*). This inhibitory effect of cortisol is reversed by prostaglandins (*114*). Cortisol might have a role in regulating the transcription of some whey protein genes; sequences bearing some resemblance to glucocorticoid receptor binding sites have been found in the 5′ flanking region of human α-lactalbumin gene (*66*), and potential binding sites for glucocorticoid receptor were also found in the 5′ flanking region of the whey acidic protein genes of rat and mouse (*115*).

Cortisol can remarkably extend the half-life of casein messenger RNA (*116*). In explants from rat mammary gland cultured in the presence of insulin, cortisol, and prolactin, glucocorticoid withdrawal reduces the half-life of casein mRNA to 1 hr and also results in the decrease of transcription of the casein gene (*117*). The effect of cortisol on casein mRNA is specific, since actin mRNA transcription occurs normally with or without the glucocorticoid (*117*). Cortisol also affects the lactogenic response of mammary tissue by regulating prolactin binding to the epithelial cells (*118*). These findings support the hypothesis of a regulatory role for glucocorticoids on the differentiation of mammary epithelium (*5,7*).

Prolactin

Prolactin is the main determinant of functional differentiation of mammary epithelium (*5,7,83*). Added to a mouse mammary gland culture in combination with insulin and glucocorticoid, prolactin induces milk protein gene expression in mammary epithelial cells. Prolactin alone is sufficient to trigger the synthesis of casein in explant culture from pregnant goats, but the efficient maintenance of the cultured tissue requires insulin and cortisol (*119*).

The responsiveness of mammary cells to prolactin can be influenced by several factors. For example, cells from midpregnant mice cultured on floating collagen gels show high responsiveness to insulin, cortisol, and prolactin and synthesize large amounts of casein for prolonged periods. Mammary epithelium from virgin mice is less sensitive to the lactogenic stimulus in culture; however, pretreatment of the virgin mouse with progesterone for 2 weeks produces a transient increase in responsiveness. A similar effect is produced by applying a pituitary allograft to increase prolactin levels (*120*). These data support the concept that the mammary cells' sensitivity to prolactin during pregnancy is regulated by progesterone and prolactin levels. Mammary fat pad cells appear not to be involved in the process of functional differentiation of the epithelium (*120*).

The influence of pregnancy, and lactation on the level of prolactin receptors in the mammary gland has been investigated in several animal species. In general, receptor levels are high in the mammary gland from virgin animal, decrease during pregnancy, and increase again after or near delivery (*121–123*). Prolactin upregulates the number of its receptors, whereas progesterone antagonizes this effect of prolactin (*124,125*). The number of prolactin receptors on mammary cells from rabbit (*122*) and mouse (*121*) in different developmental stages varies in inverse relationship to progesterone levels in serum. Glucocorticoid increases prolactin receptors in mammary cells in culture (*118,126*).

After binding of prolactin to the receptor, the complex hormone-receptor is internalized; as in the case of EGF and its receptor, internalization results in a downregula-

tion of the receptor number (*122,127,128*). It is not clear whether internalization is required for prolactin action. It appears thus that prolactin regulates its receptor level both in a positive (*129*) and in a negative (*127*) fashion.

Prolactin is not necessary for the morphological development of rough endoplasmic reticulum in mouse mammary gland, but it increases the RNA content in the membranes. However, prolactin has been shown to be necessary *in vivo* for the complete structural differentiation of mammary epithelium in cows (*130*). Prolactin depletion causes only partial development of rough endoplasmic reticulum and the cellular area occupied by Golgi apparatus decreases by 11% compared to normal (*130*). Like insulin, prolactin also appears to stimulate both ribosomal and transfer RNA accumulation; glucocorticoid prolongs this effect.

Growth hormone and placental lactogen can substitute for prolactin in epithelial cell culture of mouse mammary gland. Only at concentrations as high as 50 μg/mL can bovine growth hormone stimulate casein synthesis in goat mammary tissue in culture (*131*). Bovine growth hormone cannot mimic the effect of prolactin on DNA synthesis in goat mammary explants (*131*).

Explants from pregnant rats can synthesize α-lactalbumin in the absence of prolactin, but those from virgin animals cannot unless the rat is pretreated with progesterone or estrogen (*132*). The production of α-lactalbumin in response to prolactin has been also studied in human mammary gland culture. Apparently, α-lactalbumin synthesis responds to prolactin stimulation in normal breast but is independent (when it occurs) of prolactin in malignant tissue. These findings suggest that prolactin receptors could be somehow defective in malignant breast cells (*133*). In mouse mammary epithelial cells cultured on floating collagen gels, prolactin increases ion transport across the epithelial layer, and affects passive permeability (*134,135*).

Using several agents affecting calcium transport and distribution, Bolander showed the involvement of the calcium-calmodulin system in prolactin-induced differentiation. However, this system appears not to serve as a sole mediator of prolaction action, since the calcium ionophore A23187, which reproduces the effect of prolactin on calcium accumulation, fails to induce differentiation in terms of casein synthesis (*136*).

Very recent work with tubulin-binding drugs provided the evidence that the action of prolactin to induce casein gene expression needs integrity of the microtubules in the mammary cell (*137*). Colchicine desensitizes the mammary epithelium to prolactin action (*138*).

Estrogens

17-β-Estradiol (E2) is required *in vivo* for both ductal growth during sexual maturation and lobuloalveolar development during pregnancy (*7*). In human breast cell primary culture, E2 and progesterone act as antagonists in regulating cell multiplication (*139*): Estrogen acts as an inducer of cell proliferation; progesterone shifts the growth pattern to differentiation. In normal human breast epithelium, E2 stimulates the growth of ductal system and progesterone affects the development of acini. Mammary cell cultures from lactating rats proliferate actively in response to estradiol and progesterone at a rate similar to that of mammary epithelium during pregnancy (*19*).

Calaf et al. reported that insulin, cortisol, and 17-β-estradiol shorten the length of the cell cycle in cultured mammary cells from human normal breast and suggested that the cells could be hormonally induced to reenter the cell cycle from G_0. They also reported that estrogen can modify the length of the S phase and proposed that this would account for the increase in the rate of DNA synthesis caused by the ovarian hormone (*140*).

In explants from human mammary gland at resting stage, estriol and estradiol caused a qualitatively different profile of growth stimulation (*141*). The third naturally occurring estrogen, estrone, does not show any stimulatory activity on thymidine uptake in cell culture (*142*). Proliferating response of normal mammary epithelium to estradiol is potentiated by the presence of stromal cells (*143*).

Estradiol induces modifications in the plasma membrane of epithelial cells; for example, the number and length of microvilli are increased. This appears to be a specific effect of estradiol, as progesterone, dexamethasone, and dihydrotestosterone are ineffective (*144*).

Casein synthesis and lactose synthetase activity are stimulated by 17-β-estradiol in explant cultures of mammary gland from pregnant mice. Mammary tissue from rats that have been ovariectomized and adrenalectomized loses the capacity to synthesize casein and to accumulate the casein mRNA although it retains the ability to respond to individual hormones (*145*). These data provide the evidence for the role of estrogen in mediating the tissue sensitivity to the lactogenic stimuli.

Recently, Sheffield and co-workers found that the mammary gland growth induced by ovarian steroids is accompanied by an increase in cAMP-dependent protein kinase activity and tyrosine-kinase activity (*146*). By contrast, cGMP-dependent protein phosphorylation is decreased by progesterone but not by estrogen (*146*). Protein kinase C activity shows development-related regulation; a decrease is observed during pregnancy and throughout lactation. In addition, inhibitors of protein kinase C enhance α-lactalbumin production in explant culture with insulin, cortisol, and prolactin (*147*). *In vivo*, variations in protein kinase C activity appear to be related to the sex steroids rather than to the peptide hormones (*148*). These findings indicate that the pattern of protein phosphorylation in mammary epithelial cells undergoes substantial changes during mammary growth and differentiation, suggesting that it may represent an important regulatory step.

Epidermal Growth Factor

As described earlier, the evidence for the involvement of EGF in the development of the mammary gland has been provided by several studies. EGF stimulates proliferation of the epithelium in explants, whole gland,

and cell cultures (*70,89,149,150*). Thymidine incorporation is stimulated about 5-fold, and the number of epithelial cells increases by 30 to 40%.

Like insulin, EGF action is mediated by a receptor that has tyrosine kinase activity. Normal mammary cells have specific EGF receptors with a high and a low affinity (K_d = 0.1 nM; 3.6 nM) (*150*). The occupancy of EGF receptor for a half-maximal stimulation of DNA synthesis was about 10% of total receptors. Specific EGF binding to mammary gland membranes decreases at the beginning of gestation, then rises around day 5 to reach a maximum at day 10. During the second half of pregnancy and throughout lactation, there is a constant decrease in specific binding of EGF to the membranes. Thus EGF receptor levels are high during the proliferative phases of mammary gland development and decrease when the gland reaches functional differentiation (*39*). Whether the mitogenic activity of EGF requires the activation of tyrosine kinase is not clear.

Mouse mammary epithelial cells in culture show a spontaneous hyperpolarizing response when cultured in the presence of EGF and produce a depolarizing response when incubated with insulin (*151*). The hyperpolarizing response is mediated by activity of a Ca^{2+}-dependent K^+ channel, whereas the ionic species involved in the depolarization response have not been identified (*152*). The hyperpolarization induced by EGF occurs prior to cell proliferation and could be involved in the mitogenic action of EGF.

12-*O*-tetradecanoylphorbol-13-acetate (TPA), a potent tumor promoter (*153*), can mimic the effect of EGF on mammary cell proliferation *in vitro* (*154*). The phorbol ester can increase the specific binding of EGF to its receptors on the mammary cell (*39,150*), but it is not clear whether the stimulation occurs through an increase in the number of receptors, or rather a higher affinity of the binding. It has been proposed that TPA could inhibit the downregulation of the EGF receptors (*155*). This effect could be mediated by the TPA-induced kinase C activation. TPA can inhibit the production of milk proteins as well. TPA and EGF decrease prolactin binding to the epithelial cells by 50%.

When EGF is added to a culture with insulin, cortisol, and prolactin, it inhibits the induction of milk proteins by 50%. This effect and the stimulation of DNA synthesis are both obtained by using a physiological concentration of the growth factor (*70,150*). In addition, these effects are specific, as fibroblast growth factor, multiplication stimulating activity, nerve growth factor, and platelet-derived growth factor were ineffective. EGF has been also shown to inhibit the synthesis of k casein and its mRNA accumulation in the presence of insulin, aldosterone, cortisol, and prolactin (*156*). However, EGF can stimulate the accumulation of α-casein mRNA when prolactin is omitted in mouse mammary explants (*156*).

EGF appears to be essential for the formation of lobuloalveolar structure and for the expression of differentiative potential of the mouse mammary gland. In ovariectomized and sialoadenectomized mice, replacement therapy with ovarian steroids is not sufficient to restore the responsiveness of the tissue to the lactogenic stimuli. Moreover, the stimulatory effect of EGF is abolished by the absence of either 17-β-estradiol or progesterone, indicating a synergistic action of EGF and ovarian steroids in inducing lobuloalveolar growth and in enhancing the tissue ability to synthesize casein (*45*). Mammary explants from sialoadenectomized mice synthesize less casein in response to the lactogenic stimuli (*44*).

EGF is very likely to play an important role in the appearance of spontaneous mammary tumors in certain mouse strains. EGF concentration in the submandibular gland starts raising after 30 weeks of age, and shortly thereafter the mammary tumor incidence increases to reach a plateau at week 52. Moreover, the tumor incidence in mice that have been previously sialoadenectomized drops from 62.5 to 13% at 52 weeks of age, and the latency in the appearance of the neoplasm is shifted as much as 14 weeks. Even when sialoadenectomy was performed on tumor-bearing mice, it resulted in a rapid inhibition of tumor growth, whereas the tumor resumed its growth upon administration of EGF (*157*).

These results indicate that EGF stimulates proliferation of both normal and neoplastic growth of mammary epithelial cells. It has also been reported that EGF-like substances are produced by breast cancer cells (*158,159*). In HBL100 cells, a human mammary epithelial cell line that binds both EGF and glucocorticoids, EGF appears to enhance tyrosine phosphorylation of the glucocorticoid receptor, while dexamethasone prevents EGF effect (*160*).

Transforming Growth Factor β

TGF-β is a 25,000 molecular weight polypeptide found in cultured normal cells from connective tissues and various epithelia. TGF-β can inhibit normal and cancerous growth, but little is known about its physiological role. When it was implanted in slow-release plastic pellets into the developing mouse mammary gland, TGF-β inhibited markedly mammary growth and morphogenesis, and the inhibitory effect was fully reversible (*161*). These results suggest that TGF-β mimics a natural negative growth regulator or is itself such a regulatory agent, which antagonizes the mitogenic effect of growth factors such as EGF.

Thyroid Hormones

Thyroid hormones are not necessary for ductal growth but seem to stimulate lobular development (*7*). They are also important for maintenance of alveoli during regression of the gland (*35*).

In terms of differentiation, thyroid hormones seem to enhance the tissue responsiveness to prolactin in mouse mammary gland by activating the prolactin receptors both *in vivo* and in explant culture (*162,163*). In rabbit mammary tissue, thyroid hormone enhances prolactin-induced casein synthesis, acting probably at a posttranscriptional level (*164*). In explants from pregnant goats, neither L-T3 nor progesterone appears to influence the

synthesis of casein and total proteins stimulated by prolactin (*165*). Thyroid hormones also regulate the level of EGF receptors *in vivo* at several stages of development. Lack of thyroid activity exerts a negative effect on EGF binding to mammary epithelial membranes by decreasing the number of binding sites (*166*).

The activity of thyroid hormone-binding inhibitor (THBI), which decreases the binding of T4 to T4-binding globulin in serum, is reported to be higher in lactating rat mammary gland than in pregnant or virgin tissue (*167*). However, it remains unclear whether the higher THBI activity and the consequent increase of free T4 available to the lactating epithelium is necessary in the regulation of lactation or whether it is just a consequence of the full differentiation.

Retinoids

It has been reported that retinoids inhibit differentiation and proliferation in the mammary gland of experimental animal (*168,169*). Retinoic acid, but not retinyl acetate, can reverse the growth stimulation induced by ovarian hormones in lactating rat mammary gland in organ culture (*19*). By contrast, in mouse mammary explants, retinoic acid potentiates the stimulatory effect of epidermal growth factor on DNA synthesis and enhances the specific binding of EGF to its receptor (*170*).

Vitamin A is known for its anticarcinogenic activity in the mammary epithelium (*171,172*), but its involvement in the functional development and maintenance of the mammary gland needs to be clarified. Mammary explants from vitamin-A deficient rats show no abnormality in producing casein and α-lactalbumin under appropriate hormonal stimulation (*173*). In mammary explants from mice, retinoic acid has no effect on casein synthesis, but significantly decreases α-lactalbumin production in a dose-dependent fashion (*170*).

Progesterone alone or in combination with estrogen induces a remarkable increase in cellular retinoic acid binding protein (CRABP) *in vitro* (*174*). The actions of retinoids *in vivo* may be dependent on the available amount of CRABP in the tissue, which varies as the function of the developmental stage and physiological condition of the animal.

Vitamin D3

The addition of vitamin D3 significantly increases casein production in mouse mammary explants cultured in the presence of insulin, cortisol, and prolactin (*175*). Moreover, Bhattacharjee et al. found that mammary gland explants from rachitic rats and mice show a decreased production of milk proteins when cultured in the presence of insulin, prolactin, and glucocorticoid (*176*). Addition of 1,25-dihydroxycholecalciferol to the culture does not reverse the reduction, whereas pretreatment of the animal with the vitamin for 10 days can correct the defect. DNA synthesis is not affected by vitamin D deficiency (*176*). The action of vitamin D3 is specifically directed to the epithelial cells, that have been shown to concentrate the hormone in their nuclei (*177*). In addition, mouse mammary epithelial cells have been shown to possess specific vitamin D receptors, the level of which is enhanced by insulin, prolactin, and glucocorticoid (*178*). These data strongly suggest that vitamin D3 at physiological concentrations may be involved in the control of lactogenesis.

Some Intracellular Regulatory Agents

Calcium. Another factor affecting mammary growth in short-term cultures is Ca^{2+} concentration. Reduction of Ca^{2+} to levels below 0.08 mM leads to resumption of cell growth, but under these conditions differentiation does not occur. The effect of Ca^{2+} is specific and reversible (*179*). These findings suggest a possible modulation of growth-differentiation pathway by alterations in calcium level by such agents as phosphatidyl inositol, vitamin D3, or prolactin which affect calcium metabolism (*180*).

Polyamines. Spermidine is essential for milk protein synthesis *in vitro* (*181*); it can substitute for glucocorticoid in the induction of α-lactalbumin and, partially, casein synthesis in cultured mouse mammary tissue. Addition of methylglyoxalbis(guanylhydrazone) (MGBG), an inhibitor of polyamine biosynthesis, to the culture medium, causes the inhibition of prolactin-stimulated RNA, casein, and lipid synthesis. The inhibition is reversed by addition of exogenous spermidine (*182*).

In mammary explant cultures pretreated with insulin and cortisol, spermidine in combination with prostaglandin can mimic the action of prolactin on casein synthesis (*183*). Spermidine levels, as well as ODC activity, are elevated in response to prolactin. The requirement for spermidine for milk protein synthesis appears to be different among various species, but it is generally accepted that spermidine plays an important role in lactogenesis.

Poly(ADP-ribose). A decreased amount of poly(ADP-ribose) in the mammary gland tissue has been observed *in vivo* during pregnancy and lactation, suggesting an inverse relationship with the differentiation process (*184*). Data obtained from *in vitro* experiments offer a possible explanation for this decrease: in explant culture insulin stimulates the activity of poly(*ADP*-ribosyl)glycohydrolase, an enzyme responsible for poly(*ADP*-ribose) degradation, while prolactin appears to inhibit the specific synthetase activity. However, the role of poly(*ADP*-ribose) in mammary differentiation is unclear, as pharmacological inhibition of poly(*ADP*-ribosyl) synthetase results in an increase in α-lactalbumin accumulation (*184,185*).

Concluding Remarks

In this paper we have reviewed the recent literature related to the control of growth and differentiation of mammary gland. Experimental systems employed to assess the contribution of hormones and growth factors have been quite diverse, involving both *in vivo* and *in vitro* systems, animals from different species and strains and, more recently, several cell lines derived from normal or neoplastic tissue.

The most widely used approach in studies *in vivo* is to induce a specific hormonal deficiency and then to observe the ability of the mammary tissue to undergo the physiological changes related to pregnancy and lactation. The deficient condition may be obtained by different methods, such as surgical removal of the main hormonal source or antibodies against the hormone or its receptor. Replacement therapy can provide further information about the function of the specific factor studied. These approaches have been used in the recent study of the role of EGF in mammary gland during pregnancy.

In vitro systems have been successfully used to study the direct involvement of various hormones. It is possible to maintain mammary cells in the various culture systems using chemically defined medium and to monitor the degree of differentiation of the mammary tissue by measuring the production of milk proteins and their respective mRNA. These simplified systems have helped, at least to some extent, to clarify the contribution of the individual regulatory factors.

The mode of interactions among hormones and growth factors is different and varies with the physiological condition of the gland. Estrogens may be considered as the main effectors of mammary epithelial growth; however, the cell sensitivity to estrogen is controlled by progesterone or vice versa. In addition, recent studies have revealed the importance of EGF as mammary cell growth factor during pregnancy. It is also noteworthy that its production is regulated by ovarian steroids.

On the other hand, prolactin plays the major role in the induction of differentiation. Its receptor is regulated by some other hormones and growth factors such as insulin, glucocorticoid, and EGF. From these studies it is becoming more evident that hormone and growth factor receptors are not just passive carriers of a message but can actually modulate the signal by participating in complex regulatory circuits involving intracellular transmitters. Receptors are perhaps the main communication system through which mammary cells can adapt their behavior to the surrounding environment, and defective receptors are likely to be responsible for some kinds of mammary hyperplasia.

In the past, knowledge of the specific roles of different regulators of mammary function was mainly limited to the description of their effects on cell phenotype and cell physiology. The more powerful techniques now available allow us to focus on understanding how intracellular mediators are related to exogenous stimuli; on the other hand several current studies are trying to elucidate when and how determinants of mammary cell growth and differentiation are interacting with the processes of DNA replication, transcription and message translation.

REFERENCES

1. Nandi, S. Endocrine control of mammary gland development and function in the C3H/He Crgl mouse. J. Natl. Cancer Inst. 21: 1039-1063 (1958).
2. Lyons, W. R., Li, C. H., and Johnson, R. E. The hormonal control of mammary growth and lactation. Recent Prog. Horm. Res. 14: 219-250 (1958).
3. Cowie, A. T., and Tindal, J. S. The physiology of lactation. Monographs of the Physiological Society No. 22, Williams and Wilkins, Baltimore, MD, 1971.
4. Tucker, H. A. General endocrinological control of lactation. In: Lactation: A Comprehensive Treatise, Vol. 1 (B. L. Larson, Ed.), Academic Press, New York, 1974, pp. 277-326.
5. Banerjee, M. R. Responses of mammary cells to hormones. Int. Rev. Cytol. 47: 1-97 (1976).
6. Elias, J. J. The role of prolactin in normal mammary gland growth and function. In: Hormonal Proteins and Peptides, Vol. 8, (C. H. Li, Ed.), Academic Press, New York, 1980, pp. 37-74.
7. Topper, Y. J., and Freeman, C. S. Multiple hormone interactions in the developmental biology of the mammary gland. Physiol. Rev. 60: 1049-1106 (1980).
8. Porter, J. C. Hormonal regulation of breast development and activity. J. Invest. Dermatol. 63: 85-92 (1974).
9. Anderson R. R. Embryonic and fetal development of the mammary apparatus. In: Lactation: A Comprehensive Treatise, Vol. 4 (B. L. Larson, Ed.), Academic Press, New York, 1978, pp. 3-40.
10. Raynaud, A., and Raynaud, J. Les processus de la destruction de la deuxieme paire inguinale d'ebauches mammaires des foetus males de souris. C. R. Seances Soc. Biol. Paris 147: 1962-1967 (1953).
11. Daniel, C. W., Berger, J. J., Strickland, P., and Garcia, R. Similar growth pattern of mouse mammary epithelium cultivated in collagen matrix in vivo and in vitro. Dev. Biol. 104: 57-64 (1984).
12. Williams, J. M., and Daniel, C. W. Mammary ductal elongation: Differentiation of myoepithelium and basal lamina during branching morphogenesis. Dev. Biol. 97: 274-290 (1983).
13. Joshi, K., Smith, J. A., Perusinghe, N., and Monoghan, P. Cell proliferation in the human mammary epithelium. Differential contribution by epithelial and myoepithelial cells. Am. J. Pathol. 124: 199-206 (1986).
14. Emerman, J. T., and Vogl, A. W. Cell size and shape changes in the myoepithelium of the mammary gland during differentiation. Anat. Rec. 216: 405-415 (1986).
15. Berger, J. J., and Daniel, C. W. Stromal DNA synthesis is stimulated by young, but not serially aged, mouse mammary epithelium. Mech. Ageing Dev. 23: 277-284 (1983).
16. Bresciani, F. Effect of ovarian hormones on duration of DNA synthesis in cells of the C3H mouse mammary gland. Exp. Cell. Res. 38: 13-32 (1965).
17. Baldwin, R. L., and Lang, Y. T. Enzymatic and metabolic changes in the development of lactation. In: Lactation: A Comprehensive Treatise, Vol. 1 (B. L. Larson and V. R. Smith, Eds.), Academic Press, New York, 1974, pp. 349-411.
18. Davis, C. L., and Bauman, D. E. General metabolism associated with the synthesis of milk. In: Lactation: A Comprehensive Treatise, Vol. 2 (B. L. Larson and V. R. Smith, Eds.), Academic Press, New York, 1974, pp. 3-30.
19. Kute, T. E., Linville, C., Mehta, R. G., and Moon, R. C. Cell kinetics in normal neoplastic mammary tissue by flow cytometric analyses. Cytometry 6: 362-367 (1985).
20. Shipman, L. J., Docherty, A. H., Knight, C. H., and Wilde, C. J. Metabolic adaptations in mouse mammary gland during a normal lactation cycle and in extended lactation. Q. J. Exp. Physiol. 72: 303-311 (1987).
21. Raynaud, A. Recherches experimentales sur le developpement de l'appareil genital et le fonctionnement des glande endocrines des foetus de souris et de mulot. Arch. Anat. Microsc. Morphol. Exp. 39: 518-576 (1950).
22. Cowie, A. T. The hormonal control of milk secretion. In: Milk: The Mammary Gland and Its Secretion, Vol. 1 (S. K. Kon and A. T. Cowie, Eds.), Academic Press, New York, 1961, pp. 163-203.
23. Jacobsohn, D. Hormonal regulation of mammary gland growth. In: Milk: The Mammary Gland and Its Secretion, Vol. 1 (S. K. Kon and A. T. Cowie, Eds.), Academic Press, New York, 1961, pp. 127-160.
24. Sakakura, T., and Nishizuka, Y. Effect of thymectomy on mammary tumorigenesis, noduligenesis, and mammogenesis in the mouse. Gann 58: 441-450 (1967).
25. Oka, T., Perry, J. W., and Topper, Y. J. Changes in insulin responsiveness during development of mammary epithelium. J. Cell Biol. 62: 550-556 (1974).
26. Reece, R. P., and Leonard, S. L. Effect of estrogens, gonadotropins

and growth hormone on mammary gland of hypophysectomized rats. Endocrinology 29: 297–305 (1941).
27. Silver, M. The onset of allometric mammary growth in the female hooded Norway rat. J. Endocrinol. 10: 35–45 (1953).
28. Shyamala, G., and Ferenczy, A. Mammary fat pad may be a potential site for initiation of estrogen action in normal mouse mammary glands. Endocrinology 115: 1078–1081 (1984).
29. Sheffield, L. G., and Welsch, C. W. Cholera-toxin-enhanced growth of human breast cancer cell lines in vitro and in vivo: Interaction with estrogen. Int. J. Cancer 36: 479–483 (1985).
30. Silberstein, G. B., Strickland, P., Trumpbour, V., Coleman, S., and Daniel, C. W. In vivo, cAMP stimulates growth and morphogenesis of mouse mammary ducts. Pro. Natl. Acad. Sci. (U.S.) 81: 4950–4954 (1984).
31. Sheffield, L. G., Sinha Y. N., and Welsch, C. W. Cholera toxin treatment increases in vivo growth and development of the mouse mammary gland. Endocrinology 117: 1864–1869 (1985).
32. Lee, A. E. The proliferative action of oestriol. J. Endocrinol. 84: 289–294 (1980).
33. Traurig, H. H., and Morgan, C. F. The effect of ovarian and hypophyseal hormones on mammary gland epithelial cell proliferation. Anat. Rec. 150: 423–434 (1964).
34. Turner, C. W., and Gomez, E. T. The experimental development of the mammary gland. I. The male and female albino mouse. Mo. Agri. Exp. Stn. Res. Bull. 206: 5–16 (1934).
35. Vonderhaar, B. K., and Greco, A. E. Effect of thyroid status on development of spontaneous mammary tumors in primiparous C3H mice. Cancer Res. 42: 4553–4561 (1982).
36. Haslam, S. Z., and Shyamala, G. Relative distribution of estrogen and progesterone receptors among the epithelial, adipose, and connective tissue components of the normal mammary gland. Endocrinology 108: 825–830 (1981).
37. Haslam, S. Z., and Shyamala, G. Progesterone receptors in normal mammary glands of mice: Characterization and relationship to development. Endocrinology 105: 786–795 (1979).
38. Terada, N., Wakimoto, H., and Oka, T. Regulation of milk protein synthesis by progesterone in cultured mouse mammary gland. J. Steroid Biochem. 29: 99–104 (1988).
39. Edery, M., Pang, K., Larson, L., Colosi, T., and Nandi, S. Epidermal growth factor receptor levels in mouse mammary glands in various physiological states. Endocrinology 117: 405–411 (1985).
40. Kurachi, H., and Oka, T. Changes in epidermal growth factor concentrations of submandibular gland, plasma and urine of normal and sialoadenectomized female mice during various reproductive stages. J. Endocrinol. 106: 197–202 (1985).
41. Byyny, R. L., Orth, D. N., and Cohen, S. Epidermal growth factor: Effects of androgens and adrenergic agents. Endocrinology 95: 776–782 (1974).
42. Bullock, L., Barthe, P. L., Mowszowicz, I., Orth, D. N., and Bardin, C. W. The effect of progestins on submaxillary gland epidermal growth factor: Demonstration of androgenic, synandrogenic and antiandrogenic actions. Endocrinology 97: 189–195 (1975).
43. Barkley, M. S., Geschwind, I. I., and Bradford, G. E. The gestational pattern of estradiol, testosterone and progesterone secretion in selected strains of mice. Biol. Reprod. 20: 733–738 (1979).
44. Okamoto, S., and Oka, T. Evidence for physiological function of epidermal growth factor: Pregestational sialoadenectomy of mice decreases milk production and increases offspring mortality during lactation period. Proc. Natl. Acad. Sci. (U.S.) 81: 6059–6063 (1984).
45. Oka, T., Tsutsumi, O., Kurachi, H., and Okamoto, S. The role of epidermal growth factor in normal and neoplastic growth of mouse mammary epithelial cells. In: Breast Cancer: Cellular and Molecular Biology (M. E. Lippman and R. Dickson, Eds.), Kluwer Academic Publishers, 1988, pp. 343–362.
46. Daniel C. W., Silberstein, G. B., and Strickland, P. Reinitiation of growth in senescent mouse mammary epithelium in response to cholera toxin. Science 224: 1245–1247 (1984).
47. Kratochwil, K. Development and loss of androgen responsiveness in the embryonic rudiment of the mouse mammary gland. Dev. Biol. 61: 358–365 (1977).
48. Heuberger, B., Fitzka, I., Wasner, G., and Kratochwil, K. Induction of androgen receptor formation by epithelium-mesenchyme interaction in embryonic mouse mammary gland. Dev. Biol. 97: 274–290 (1983).
49. Wasner, G., Hennerman, I., and Kratochwil, K. Ontogeny of mesenchymal androgen receptors in the embryonic mouse mammary gland. Endocrinology 113: 1771–1778 (1983).
50. Hoshino, H. Morphogenesis and growth potentiality of mammary glands in mice. I. Transplantability and growth potentiality of mammary tissue in virgin mice. J. Natl. Cancer Inst. 29: 835–851 (1962).
51. Slavin, B. Growth of mammary transplants in various tissues and organ sites in the mouse (abstr.). Anat. Rec. 154: 423 (1966).
52. Levine, J. F., and Stockdale, F. E. Cell-cell interactions promote mammary epithelial cell differentiation. J. Cell. Biol. 100: 1415–1422 (1985).
53. Wakimoto, H., and Oka, T. Involvement of collagen formation in the hormonally induced functional differentiation of mouse mammary gland in organ culture. J. Biol. Chem. 258: 3775–3779 (1983).
54. Enami, J., and Nandi, S. Hormonal control of milk protein synthesis in cultured mouse mammary explants. Cell Differ. 6: 217–227 (1977).
55. Ono, M., and Oka, T. The differential actions of cortisol on the accumulation of α-lactalbumin and casein in mid-pregnant mouse mammary gland in culture. Cell 19: 473–480 (1980).
56. Takemoto, T., Nagamatsu, Y., and Oka, T. Casein and α-lactalbumin messenger RNAs during the development of mouse mammary gland: Isolation, partial purification and translation in a cell-free system. Dev. Biol. 78: 247–257 (1980).
57. Fitzgerald, D. K., Colvin, B., Mawal, R., and Ebner, K. E. Enzymatic assay for galactosyltransferase activity of lactose synthetase and α-lactalbumin in purified and crude systems. Anal. Biochem. 36: 43–61 (1970).
58. Hori, C., and Oka, T. Induction by lithium ion of multiplication of mouse mammary epithelium in culture. Proc. Natl. Acad. Sci. (U.S.) 76: 2823–2827 (1979).
59. Nagamatsu, Y., and Oka, T. Purification and characterization of mouse α-lactalbumin and preparation of its antibody. Biochem. J. 185: 227–237 (1980).
60. Schultz, G. S., and Ebner, K. E. Measurement of α-lactalbumin in serum and mammary tumor of rats by radioimmunoassay. Cancer Res. 37: 4482–4488 (1977).
61. Richards, D. A., Rodgers, J. R., Supowit, S. C., and Rosen, J. M. Construction and preliminary characterization of the rat casein and alpha-lactalbumin cDNA clones. J. Biol. Chem. 256: 526–532 (1981).
62. Hennighausen, L. G., Steudle, A., and Sippel, A. E. Nucleotide sequence of cloned cDNA coding for mouse epsilon casein. Eur. J. Biochem. 126: 569–572 (1982).
63. Hennighausen, L. G., and Sippel, A. E. Characterization and cloning of the mRNAs specific for the lactating mouse mammary gland. Eur. J. Biochem. 125: 131–141 (1982).
64. Hennighausen, L. G., and Sippel, A. E. Comparative sequence analysis of the mRNAs coding for mouse and rat whey protein. Nucl. Acid Res. 10: 3733–3744 (1982).
65. Yoshimura, M., Banerjee, M. R., and Oka, T. Nucleotide sequence of a cDNA encoding mouse beta casein. Nucl. Acid Res. 14: 8224 (1986).
66. Hall, L., Emery, D. C., Davies, M. S., Parker, D., and Craig, R. K. Organization and sequence of the human α-lactalbumin gene. Biochem. J. 242: 735–742 (1987).
67. Yu-Lee, L., Richter-Mann, L., Couch, C. H., Stewart, A. F., Mackinlay, A. G., and Rosen, J. M. Evolution of the casein multigene family: Conserved sequences in the 5′ flanking and exon regions. Nucl. Acid Res. 14: 1883–1902 (1986).
68. Elias, J. J. Cultivation of adult mouse mammary gland in hormone-enriched synthetic medium. Science 126: 842–844 (1957).
69. Ichinose, R. R., and Nandi, S. Lobuloalveolar differentiation in mouse mammary tissue in vitro. Science 145: 496–497 (1964).
70. Taketani, Y., and Oka, T. Epidermal growth factor stimulates cell proliferation and inhibits functional differentiation of mouse mammary epithelial cells in culture. Endocrinology 113: 871–877 (1983).
71. Yang, J., Richards, J., and Bowman, P., Guzman, R., Enami, J., McCormick, K., Hamamoto, S., Pitelka, D., and Nandi, S. Sustained growth and three-dimensional organization of primary mammary tumor epithelial cells embedded in collagen gels. Proc. Natl. Acad. Sci. (U.S.) 76: 3401–3405 (1979).
72. Yang, N. S., Kube, D., Park C., and Furmanski P. Growth of human

mammary epithelial cells on collagen gel surfaces. Cancer Res. 41: 4093–4100 (1981).
73. Yang, J., Guzman, R., Richards, J., Jentoft, V., DeVault, M. R., Wellings, S. R., and Nandi, S. Primary culture of human mammary epithelial cells embedded in collagen gels. JNCI 65: 337–343 (1980).
74. Emerman, J. T., and Pitelka, D. R. Maintenance and induction of morphological differentiation in dissociated mammary epithelium on floating collagen membranes. In Vitro 13: 316–328 (1977).
75. Imagawa, W., Tomooka, Y., and Nandi, S. Serum-free growth of normal and tumor mouse mammary epithelial cells in primary culture. Proc. Natl. Acad. Sci. (U.S.) 79: 4074–4077 (1982).
76. Wicha, M. S., Lowrie, G., Kohn, E., Bagavandoss, P., and Mahn, T. Extracellular matrix promotes mammary epithelial growth and differentiation in vitro. Proc. Natl. Acad. Sci. (U.S.) 79: 3213–3217 (1982).
77. Wicha, M. S. Interaction of rat mammary epithelium with extracellular matrix components. Prog. Clin. Biol. Res. 145: 129–142 (1984).
78. Levine, J. F., and Stockdale, F. E. 3T3-L1 adipocytes promote the growth of mammary epithelium. Exp. Cell. Res. 151: 112–122 (1984).
79. Hardy, M. H. The development in vitro of the mammary glands of the mouse. J. Anat. London 84: 388–393 (1950).
80. Oka, T., and Topper, Y. J. Hormone-dependent accumulation of rough endoplasmic reticulum in mouse mammary epithelial cells in vitro. J. Biol. Chem. 246: 7701–7707 (1971).
81. Devinoy, E., Houdebine, L. M., and Ollivier-Bousquet, M. Role of glucocorticoids and progesterone in the development of rough endoplasmic reticulum involved in casein biosynthesis. Biochemie 61: 453–461 (1979).
82. Mills, E. S., and Topper, Y. J. Some ultrastructural effects of insulin, hydrocortisone and prolactin on mammary gland explants. J. Cell Biol. 44: 310–328 (1970).
83. Rosen, J. M., Matusik, R., Richard, D. A., Gupta, P., and Rogers, J. R. Multihormonal regulation of casein gene expression at the transcriptional and post-transcriptional levels in the mammary glands. Recent Prog. Horm. Res. 36: 157–193 (1980).
84. Delouis, C., and Combaud, M.-L. Lack of mitotic effects of insulin during synthesis of casein induced by prolactin in pseudopregnant rabbit mammary gland organ cultures. J. Endocrinol. 72: 393–394 (1977).
85. du Bois, M., and Elias, J. J. Subpopulations of cells in immature mouse mammary gland as detected by proliferative responses to hormones in organ culture. Dev. Biol. 106: 70–75 (1984).
86. Ichinose, R. R., and Nandi, S. Influence of hormones on lobuloalveolar differentiation of mouse mammary glands in vitro. J. Endocrinol. 35: 331–340 (1966).
87. Wood, B. G., Washburn, L. L., Mukherjee, A. S., and Banerjee, M. R. Hormonal regulation of lobuloalveolar growth, functional differentiation and regression of whole mouse mammary gland in organ culture. J. Endocrinol. 65: 1–6 (1975).
88. Dilley, W. G., and Nandi, S. Rat mammary gland differentiation in vitro in the absence of steroids. Science 161: 59–60 (1968).
89. Tonelli, Q. J., and Sorof, S. Epidermal growth factor requirement for development of cultured mammary gland. Nature 285: 250–252 (1980).
90. Ceriani, R. L. Fetal mammary gland differentiation in vitro in response to hormones. II. Biochemical findings. Dev. Biol. 21: 530–546 (1970).
91. Voytovich, A. E., and Topper, Y. J. Hormone-dependent differentiation of immature mouse mammary gland in vitro. Science 158: 1326–1327 (1967).
92. Vonderhaar, B. K., and Topper, Y. J. A role of the cell cycle in hormone-dependent differentiation. J. Cell Biol. 63: 707–712 (1974).
93. Richards, J., Hamamoto, S., Smith, S., Pasco, D., Guzman, R., and Nandi, S. Response of end bud cells from immature rat mammary gland to hormones when cultured in collagen gel. Exp. Cell Res. 147: 95–109 (1983).
94. Katiyar, V. N., Enami, J., and Nandi, S. Effect of polypeptide hormones on stimulation of casein secretion by mouse mammary epithelial cells grown on floating collagen gels. In Vitro 14: 771–774 (1978).
95. Salomon, D. S., Liotta, L. A., and Kidwell, W. R. Differential responses to growth factor by rat mammary epithelium plated on different collagen substrata in serum-free medium. Proc. Natl. Acad. Sci. (U.S.) 78: 382–386 (1981).
96. McGrath, M., Palmer, S., and Nandi, S. Differential response of normal rat mammary epithelial cells to mammogenic hormones and EGF. J. Cell Physiol. 125: 182–191 (1985).
97. Strum, J. M., and Hillman, E. A. Human breast epithelium in organ culture: effect of hormones on growth and morphology. In Vitro 17: 33–43 (1981).
98. Hillman, E. A., Valerio, M. G., Halter, S. A., Barrett-Boone, L. A., and Trump, B. F. Long-term explant culture of normal mammary epithelium. Cancer Res. 43: 245–257 (1983).
99. Hammond, S. L., Ham, R. G., and Stampfer, M. R. Serum-free growth of human mammary epithelial cells: Rapid clonal growth in defined medium and extended serial passage with pituitary extract. Proc. Natl. Acad. Sci. (U.S.) 81: 5435–5439 (1984).
100. Stampfer, M., Hallowes, R. C., and Hackett, A. J. Growth of normal human mammary cells in culture. In Vitro 16: 415–425 (1980).
101. Yang, J., Balakrishnan, A., Hamamoto, S., Beattie, C. W., Gupta, T. K., Wellings, S. R., and Nandi, S. Different mitogenic and phenotypic responses of human breast epithelial cells grown in two versus three dimensions. Exp. Cell. Res. 167: 563–569 (1986).
102. McGrath, C. M., and Soule, H. D. Renewal inhibition of human mammary cell growth in vitro: Cortisol and the recruitment of cells to terminal differentiation. J. Cell. Physiol. 116: 385–396 (1983).
103. Friedberg, S. H., Oka, T., and Topper, Y. J. Development of insulin sensitivity by mouse mammary gland in vitro. Proc. Natl. Acad. Sci. (U.S.) 67: 1493–1500 (1970).
104. Juergens, W. F., Stockdale, F. E., Topper, Y. J., and Elias, J. J. Hormone-dependent differentiation of mammary gland in vitro. Proc. Natl. Acad. Sci. (U.S.) 54: 629–634 (1965).
105. Stockdale, F. E., and Topper, Y. J. The role of DNA synthesis and mitosis in hormone-dependent differentiation. Proc. Natl. Acad. Sci. (U.S.) 56: 1283–1289 (1966).
106. Ptashne, K., Stockdale, F. E., and Conlon, S. Initiation of DNA synthesis in mammary epithelium and mammary tumors by lithium ions. J. Cell. Physiol. 103: 41–46 (1980).
107. Hori, C., and Oka, T. Vanadate enhances the stimulatory action of insulin on DNA synthesis in cultured mouse mammary gland. Biochim. Biophys. Acta 610: 235–240 (1980).
108. Turkington, R. W. The role of epidermal growth factor in mammary gland development in vitro. Exp. Cell. Res. 57: 79–85 (1969).
109. Majumder, G. C., and Turkington, R. W. Stimulation of mammary epithelial cell proliferation in vitro by protein factor(s) present in the serum. Endocrinology 88: 1506–1510 (1971).
110. Inagaki, Y., and Kohmoto, K. Changes in Scatchard plots for insulin binding to mammary epithelial cells from cycling, pregnant, and lactating mice. Endocrinology 110: 176–182 (1982).
111. Chou, C. K., Dull, T. J., Russell, D. S., Gherzi, R., Lebwohl, D., Ullrich, A., and Rosen, O. M. Human insulin receptors mutated at the ATP binding site lack protein tyrosine kinase activity and fail to mediate postreceptor effects of insulin. J. Biol. Chem. 262: 1842–1847 (1987).
112. Chomczynski, P., Qasba, P., and Topper, Y. J. Essential role of insulin in transcription of the rat 25,000 molecular weight casein gene. Science 226: 1326–1328 (1984).
113. Nagamatsu, Y., and Oka, T. Differential action of cortisol on the synthesis, turnover of α-lactalbumin and casein and their mRNA accumulation in cultured mammary gland from midpregnant mice. Biochem. J. 212: 509–515 (1983).
114. Terada, N., Ono, M., Nagamatsu, Y., and Oka, T. The reversal of cortisol-induced inhibition of α-lactalbumin by prostaglandins in the mouse mammary gland in culture. J. Biol. Chem. 257: 11199–11202 (1982).
115. Campbell, S. M., Rosen, J. M., Hennighausen, L. G., Strech-Jurk, U., and Sippel, A. E. Comparison of the whey acidic protein genes of the rat and mouse. Nucl. Acids Res. 12: 8685–8697 (1984).
116. Banerjee, M. R., Terry, P. M., Sakai, S., Lin, F. K., and Ganguly, R. Hormonal regulation of casein messenger RNA (mRNA). In Vitro 14: 128–139 (1978).
117. Chomczynski, P., Qasba, P., and Topper, Y. J. Transcriptional and post-transcriptional roles of glucocorticoid in the expression of the rat 25,000 molecular weight casein gene. Biochem. Biophys. Res. Commun. 134: 812–818 (1986).
118. Sakai, S., Bowman, P. D., Yang, J., McCormick, K., and Nandi, S.

Glucocorticoid regulation of prolactin receptors on mammary cells in culture. Endocrinology 104: 1447–1449 (1979).
119. Skarda, J., Urbanova, E., Housdebine, L. M., Delouis, C., and Bilek, J. Effects of insulin, cortisol and prolactin on lipid, protein and casein syntheses in goat mammary tissue in organ culture. Reprod. Nutr. Dev. 22: 379–386 (1982).
120. Bolander, F. F., Jr. Enhanced endocrine sensitivity in mouse mammary glands: hormonal requirements for induction and maintenance. Endocrinology 115: 630–633 (1984).
121. Sakai, S., Enami, J., Nandi, S., and Banerjee, M. R. Prolactin receptor on dissociated mammary epithelial cells at different stages of development. Mol. Cell. Endocrinol. 12: 285–298 (1978).
122. Suard, Y. M., Kraehenbuhl, J.-P., and Aubert, M. L. Dispersed mammary epithelial cells. Receptors of lactogenic hormones in virgin, pregnant, and lactating rabbits. J. Biol. Chem. 254: 10466–10475 (1979).
123. McNeilly, A. S., and Friesen, H. G. Binding of prolactin to the rabbit mammary gland during pregnancy. J. Endocrinol. 74: 507–508 (1977).
124. Bohnet, G. H., Gomez, F., and Friesen, H. G. Prolactin and estrogen binding sites in the mammary gland of the lactating and nonlactating rat. Endocrinology 101: 1111–1121 (1977).
125. Djiane, J., and Durand, P. Prolactin-progesterone antagonism in self regulation of prolactin receptors in the mammary gland. Nature 266: 641–643 (1977).
126. Taketani, Y., and Oka, T. Hormonal regulation of the synthesis of casein and α-lactalbumin in a primary mammary cell culture system. Horm. Metab. Res. 18: 119–125 (1986).
127. Djiane, J., Clauser, H., and Kelly, P. A. Rapid down-regulation of prolactin receptors in mammary gland and liver. Biochem. Biophys. Res. Commun. 90: 1371–1378 (1979).
128. Djiane, J., Houdebine, L. M., and Kelly, P. A. Correlation between prolactin-receptor interaction, down-regulation of receptors, and stimulation of casein and deoxyribonucleic acid biosynthesis in rabbit mammary gland explants. Endocrinology 110: 791–795 (1982).
129. Sheth, N. A., Tikekar, S. S., Ranadive, K. J., and Sheth, A. R. Influence of bromoergocryptine on estrogen-modulated prolactin receptors of mouse mammary gland. Mol. Cell. Endocrinol. 12: 167–176 (1978).
130. Akers, R. M., Bauman, D. E., Goodman, G. T., Capuco, A. V., and Tucker, H. A. Prolactin regulation of cytological differentiation of mammary epithelial cells in periparturient cows. Endocrinology 109: 31–40 (1981).
131. Skarda, J., Urbanova, E., Becka, S., Houdebine, L. M., Delouis, C., Pichova, D., Picha, J., and Bilek, J. Effect of bovine growth hormone on development of goat mammary tissue in organ culture. Endocrinol. Exp. (Bratisl.) 16: 19–31 (1982).
132. Nicholas, K. R., Sankaran, L., and Topper, Y. J. The induction of alpha-lactalbumin in rat mammary explants in the absence of exogenous prolactin: effects of progesterone and estrogen. Endocrinology 109: 978–980 (1981).
133. Wilson, G. D., Woods, K. L., Walker, R. A., and Howell, A. Effect of prolactin on lactalbumin production by normal and malignant human breast tissue in organ culture. Cancer Res. 40: 486–489 (1980).
134. Bisbee, C. A. Prolactin effects on ion transport across cultured mouse mammary epithelium. Am. J. Physiol. 240: C110–C115 (1981).
135. Bisbee, C. A. Transepithelial electrophysiology of cultured mouse mammary epithelium: Sensitivity to prolactins. Am. J. Physiol. 241: E410–E413 (1981).
136. Bolander, F. F., Jr. Possible roles of calcium and calmodulin in mammary gland differentiation in vitro. J. Endocrinol. 104: 29–34 (1985).
137. Servely, J. L., Geuens, G. M., Martel, P., Houdebine, L. M., and de Brabander, M. Effect of tubulozole, a new synthetic microtubule inhibitor, on the induction of casein gene expression by prolactin. Biol. Cell 59: 121–127 (1987).
138. Akers, R. M. Lactogenic hormones: binding sites, mammary growth, secretory cell differentiation, and milk biosynthesis in ruminants. J. Dairy Sci. 68: 501–519 (1985).
139. Mauvais-Jarvis, P., Kuttenn, F., Gompel, A., Malet, C., and Fournier, S. Estradiol-progesterone interaction in normal and pathological human breast cells. Ann. Endocrinol. (Paris) 47: 179–187 (1986).
140. Calaf, G., Russo, I. H., Roi, L. D., and Russo, J. Effects of peptides and steroid hormones on cell kinetic parameters of normal human breast tissue in organ culture. In Vitro Cell. Dev. Biol. 22: 135–140 (1986).
141. van Bogaert, L. J., Quinones, J., and Craynest, M. P. Estriol-induced dose-dependent DNA synthesis in normal human mammary epithelium in vitro. Eur. J. Cell Biol. 21: 234–236 (1980).
142. van Bogaert, L. J., van Craynest, M. P., and Abarca-Quinones, J. Direct influence of the three natural estrogens on human mammary gland in vitro. Horm. Metab. Res. 14: 598–601 (1982).
143. McGrath, C. M. Augmentation of the response of normal mammary epithelial cells to estradiol by mammary stroma. Cancer Res. 43: 1355–1360 (1983).
144. Chambon, M., Cavalie-Barthez, G., Veith, F., Vignon, F., Hallowes, R., and Rochefort, H. Effect of estradiol on nonmalignant human mammary cells in primary culture. Cancer Res. 44: 5733–5743 (1984).
145. Sankaran, L., Qasba, P., and Topper, Y. J. Effects of estrogen-depletion on rat casein gene expression. Biochem. Biophys. Res. Commun. 125: 682–689 (1984).
146. Sheffield, L. G., Ayslworth, C. F., and Welsch, C. W. Cyclic nucleotides and protein phosphorylation in mouse mammary glands: effects of estrogen and progesterone administered in vivo. Proc. Soc. Exp. Biol. Med. 185: 283–290 (1987).
147. Caulfield, J. J., and Bolander, F. F., Jr. Involvement of protein kinase C in mouse mammary gland development. J. Endocrinol. 109: 29–34 (1986).
148. Holladay, C. S., and Bolander, F. F., Jr. Hormonal regulation of protein kinase C in the mouse mammary gland. J. Cell Physiol. 131: 190–199 (1987).
149. Yang, J., Guzman, R., Richards, J., Imagawa, N., McCormick, K., and Nandi, S. Growth factor and cyclic nucleotide-induced proliferation of normal and malignant mammary epithelial cells in primary culture. Endocrinology 107: 35–41 (1980).
150. Taketani, Y., and Oka, T. Biological action of epidermal growth factor and its functional receptors in normal mammary epithelial cells. Proc. Natl. Acad. Sci. (U.S.) 80: 2647–2650 (1983).
151. Enomoto, K., Cossu, M. F., Edwards, C., and Oka, T. Induction of distinct types of spontaneous electrical activities in mammary epithelial cells by epidermal growth factor and insulin. Proc. Natl. Acad. Sci. (U.S.) 83: 4754–4758 (1986).
152. Enomoto, K., Cossu, M. F., Maeno, T., Edwards, C., and Oka, T. Involvement of the Ca^{++}-dependent K^+ channel activity in the hyperpolarizing response induced by epidermal growth factor in mammary epithelial cells. FEBS Lett. 203: 181–184 (1986).
153. Boutwell, R. K. The function and mechanism of promoters of carcinogenesis. CRC Crit. Rev. Toxicol. 2: 419–443 (1974).
154. Taketani, Y., and Oka, T. Tumor promoter 12-O-tetradecanoylphorbol 13-acetate, like epidermal growth factor, stimulates cell proliferation and inhibits differentiation of mouse mammary epithelial cells in culture. Proc. Natl. Acad. Sci. (U.S.) 80: 1646–1649 (1983).
155. Gordon, P., Carpenter, J. L., Cohen, S., and Orchi, L. Epidermal growth factor: Morphological demonstration of binding, internalization, and lysosomal association in human fibroblasts. Proc. Natl. Acad. Sci. (U.S.) 75: 5025–5029 (1978).
156. Vonderhaar, B. K., and Nakhasi, H. L. Bifunctional activity of epidermal growth factor on alpha and kappa casein gene expression in rodent mammary glands in vitro. Endocrinology 119: 1178–1184 (1986).
157. Kurachi, H., Okamoto, S., and Oka. T. Evidence for the involvement of the submandibular gland epidermal growth factor in mouse mammary tumorigenesis. Proc. Natl. Acad. Sci. (U.S.) 82: 5940–5943 (1985).
158. Salomon, D. S., Zwiebel, J. A., Bano, M., Losoczy, I., Fehnel, P., and Kidwell, W. R. Presence of transforming growth factors in human breast cancer cells. Cancer Res. 44: 4069–4099 (1984).
159. Dickson, R. B., Huff, K. K., Spencer, E. M., and Lippman, M. E. Induction of epidermal growth factor-related polypeptides by 17 estradiol in MCF-7 human breast cancer cells. Endocrinology 118: 138–142 (1985).
160. Rao, K. V., and Fox, C. F. Epidermal growth factor stimulates tyrosine phosphorylation of human glucocorticoid receptor in cultured cells. Biochem. Biophys. Res. Commun. 144: 512–519 (1987).
161. Silberstein, G. B., and Daniel, C. W. Reversible inhibition of mam-

mary gland growth by transforming growth factor-β. Science 237: 291-293 (1987).
162. Bhattacharya, A., and Vonderhaar, B. K. Thyroid hormone regulation of prolactin binding to mouse mammary glands. Biochem. Biophys. Res. Commun. 88: 1405-1411 (1979).
163. Vonderhaar, B. K., Bhattacharya, A., Alhadi, T., Liscia, D. S., Andrew, E. M., Young, J. K., Ginsburg, E., Bhattacharjee, M., and Horn, T. M. Isolation, characterization, and regulation of the prolactin receptor. J. Dairy Sci. 68: 466 (1985).
164. Houdebine, L. M., Delouis, C., and Devinoy, E. Post-transcriptional stimulation of casein synthesis by thyroid hormone. Biochimie 60: 735-741 (1978).
165. Skarda, J., Urbanova, E., Houdebine, L. M., Delouis, C., and Bilek, J. Hormonal control of casein synthesis in mammary explants from pregnant goats. Endokrinologie 79: 301-307 (1982).
166. Vonderhaar, B. K., Tang, E., Lyster, R. R., and Nascimento, M. C. S. Thyroid hormone regulation of epidermal growth factor receptor levels in mouse mammary glands. Endocrinology 119: 580-585 (1986).
167. Oberkotter, L. V., Farmer, L. C., and Farber, M. Thyroid hormone-binding inhibitor in normal, pregnant, and lactating rat and postmenopausal human breast tissue. Endocrinology 117: 511-514 (1985).
168. Moon, R. C., and Mehta, R. G. Anticarcinogenic effects of retinoids in animals. Adv. Exp. Med. Biol. 206: 399-411 (1986).
169. Chakraborty, S., Menon, R., and Banerjee, M. R. Influence of some dietary chemopreventive agents on the expression of functional differentiation of the mouse mammary gland in vitro. Int. J. Cancer 39: 752-759 (1987).
170. Komura, H., Wakimoto, H., Chen, C. F., Terakawa, N., Aono, T., Tanizawa, O., and Matsumoto, K. Retinoic acid enhances cell responses to epidermal growth factor in mouse mammary gland in culture. Endocrinology 118: 1530-1536 (1986).
171. Zile, M. H., Cullum, M. E., Roltsch, I. A., DeHoog, J. V., and Welsch, C. W. Effect of moderate vitamin A supplementation and lack of dietary vitamin A on the development of mammary tumors in female rats treated with low carcinogenic dose levels of 7,12-dimethylbenz(a)anthracene. Cancer Res. 46: 3495-3503 (1986).
172. Chatterjee, M., and Banerjee, M. R. N-Nitrosodiethylamine-induced nodule-like alveolar lesion and its prevention by a retinoid in BALB/c mouse mammary glands in the whole organ in culture. Carcinogenesis 3: 801-804 (1982).
173. Sankaran, L., and Topper, Y. J. Effect of vitamin A deprivation on maintenance of rat mammary tissue and on the potential of the epithelium for hormone-dependent milk protein synthesis. Endocrinology 111: 1061-1067 (1982).
174. Mehta, R. G., and Moon, R. C. Role of hormones on the induction of retinoic acid binding protein in mouse mammary gland organ culture. Carcinogenesis 6: 1103-1107 (1985).
175. Mezzetti, G., Barbiroli, B., and Oka, T. 1,25-Dihydroxycholecalciferol in hormonally-induced differentiation on mouse mammary gland in culture. In: Vitamin D. A Chemical, Biochemical and Clinical Update (A. W. Norman, K. Schaefer, H. G. Grigoleit, and D. V. Herrath, Eds.), W. de Gruyter Co., Berlin, 1985, p. 233.
176. Bhattacharjee, M., Wientroub, S., and Vonderhaar, B. K. Milk protein synthesis by mammary glands of vitamin D-deficient mice. Endocrinology 121: 865-874. (1987).
177. Narbaitz, R., Sar, M., Stumpf, W. E., Huang, S., and DeLuca, H. F. 1,25-Dihydroxyvitamin D3 target cells in rat mammary gland. Horm. Res. 15:263-269 (1981).
178. Mezzetti, G., Barbiroli, B., and Oka, T. 1,25-Dihydroxycholecalciferol receptor regulation in hormonally induced differentiation of mouse mammary gland in culture. Endocrinology 120: 2488-2493 (1987).
179. McGrath, C. M., and Soule, H. D. Calcium regulation of normal human mammary epithelial cell growth in culture. In Vitro 20: 652-662 (1984).
180. Rillema, J. A. Mechanism of action of prolactin. Fed. Proc. 39: 127-132 (1980).
181. Oka, T., Perry, J. W., and Terada, N. The regulatory function of spermidine in hormonal control of the development of mouse mammary gland in culture. Fed. Proc. 41: 3073-3077 (1982).
182. Rillema, J. A., Linebaugh, B. E., and Mulder, J. A. Regulation of casein synthesis by polyamines in mammary gland explants of mice. Endocrinology 100: 529-536 (1977).
183. Rillema, J. A. Activation of casein synthesis by prostaglandin plus spermidine in mammary gland explants of mice. Biochem. Biophys. Res. Commun. 70: 45-49 (1976).
184. Bolander, F. F., Jr. The relationship between adenosine diphosphate-ribosylation and mammary gland differentiation. J. Cell Biochem. 29: 262-372 (1985).
185. Bolander, F. F., Jr. The interrelationships among poly(ADP-ribosyl) ation, DNA synthesis and mammary gland differentiation. Biochem. Biophys. Res. Commun. 137: 359-363 (1986).